

In the ClaimsWe claim:

Claim 1 (Currently amended): A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, the method comprising the steps of:

(i) ~~reverse transcription of transcribing~~ the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each primer sequence is unique, and either or each heel sequence includes a RNA polymerase promoter site, and the FAP includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence; and

(ii) ~~amplification of amplifying~~ the cDNA using primers sufficiently complementary to the primers, *i.e.*, ~~primer sequences~~ FAP and TAP, within FAP-RAND and TAP-RT.

Claim 2 (Currently amended): ~~A-The~~ method according to claim 1, which additionally comprises the step of:

(iii) *in vitro* ~~transcription~~ transcribing, to produce RNA run-offs from either end of the amplicons.

Claim 3 (Currently amended): ~~A-The~~ method according to claim 1 ~~or claim 2~~, wherein each heel sequence includes a different RNA polymerase site.

Claim 4 (Currently amended): ~~A-The~~ method according to claim 3, for the production of a strand-specific library.

Claim 5 (Currently amended): ~~A-The~~ method according to ~~any preceding claim~~ claim 1, for the production of a subtracted library from two cell populations.

Claim 6 (Currently amended): A-The method according to any preceding claim claim 1, which further comprises cloning the polynucleotide products and immobilizing them in an array.

Claim 7 (Currently amended): A-The method according to any preceding claim claim 1, wherein the sample is from laser capture microdissection.

Claim 8 (Currently amended): A-The method according to any preceding claim claim 1, wherein the sample is from patch clamp harvesting.

Claim 9 (Currently amended): A-The method according to any preceding claim claim 1, wherein the first-and/or or the second heel sequence, or both, includes the nucleotide sequence of a cleavage site.

Claim 10 (Currently amended): A-The method according to claim 9, wherein the cleavage site is located at the 3' end of its heel sequence.

Claim 11 (Currently amended): A-The method according to claim 10, wherein the first and second heeled primers have identical cleavage sites.

Claim 12 (Currently amended): A-The method according to claim 10, wherein the first and second heeled primers have different cleavage sites.

Claim 13 (Currently amended): A-The method according to any of claims 9 to 12 claim 2, which comprises the additional step of treating the polynucleotides with an agent that cleaves at the cleavage site.

Claim 14 (Currently amended): A-The method according to any preceding claim claim 1, wherein amplification said amplifying comprises up to 50 amplification cycles.

Claim 15 (Currently amended): A-The method according to claim 14, wherein each amplification cycle comprises the steps of:

- (i) obtaining single-stranded DNA molecules at a temperature between 85°C and 97°C;

(ii) annealing the single-stranded DNA molecules at a temperature between 45°C and 65°C; and

(iii) elongating the annealed DNA molecules at a temperature between 70°C and 75°C.

Claim 16 (Currently amended): ~~A-The~~ method according to any preceding claim claim 1, wherein the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end:

(i) a heel sequence, of 15 to 22 nucleotides, which is not complementary to the mRNA molecules initially present in the sample; and

(ii) an oligo dT sequence of 15 to 25 nucleotides;

wherein substantially every possible variable sequence combination is found in said first heeled primer population.

Claim 17 (Currently amended): ~~A-The~~ method according to any preceding claim claim 1, which additionally comprises confirming the presence of at least one nucleic acid sequence contained in the reaction mixture after amplification said amplifying.

Claim 18 (Currently amended): ~~A-The~~ method according to claim 17, wherein the said confirming comprises any one of of the following methods:

(i) ~~detection of~~ detecting sequences of interest with specific oligonucleotide probes;

(ii) ~~amplification of~~ amplifying sequences of interest with specific oligonucleotide primers; and

(iii) cloning ~~of~~ the DNA molecules obtained in a replication and/or or expression vector.